I and II where $k \neq 0$ or $n \neq 2$ are the similitudes. For by (17), $Y' = \pm i$ renders $\overline{H}$ infinite. Then by the last of equations (13), the pre-images of the minimal lines $Y' = \pm i$ must make H infinite. But we know by (17) that $y' = \pm i$ are infinite roots of H. Thus the minimal lines $y' = \pm i$ must be converted into the minimal lines $Y' = \pm i$ by the collineation (2) (unless the field of force has constant slope $\pm i$ which possibility is excluded from consideration). Therefore any such collineation is necessarily a similitude.

As it is evident that similitudes do preserve the systems S_k, it follows that the proof of the Theorem of Section 5 is complete.

[1] Appell, *Amer. Jour. Math.*, 1889. The importance of the conformal group in dynamics (conservative fields of force) has been emphasized by Larmor, Goursat and Darboux.

[2] Kasner, *Differential-Geometric Aspects of Dynamics*, Princeton Colloquium Lectures, American Mathematical Society Publications 1913, 1934.

[3] Kasner, "Physical Curves," these PROCEEDINGS, **33**, 346–351 (1947).

[4] Kasner, "The Trajectories of Dynamics," *Trans. Amer. Math. Soc.*, **7**, 401–424 (1906).

THE ENERGY SOURCE FOR BIOLUMINESCENCE IN AN ISOLATED SYSTEM

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When luminous organisms are extracted with water or saline solutions one usually obtains in the crude preparation a temporary emission of light which disappears rapidly. In all but five of some twenty-one groups of luminous forms the light-emitting system is destroyed by the extraction procedures. Some of the difficulties are exemplified by the system which has been extracted from the crustacean, *Cypridina hilgendorfii*. Here, Harvey[1] demonstrated that the substrate for the reaction, luciferin, was highly unstable in the presence of air. The oxidation which occurred, however, could be reversed by simple reductants provided the compound did not remain in the oxidized state for too long a period. In the presence of the enzyme, luciferase, however, an oxidation occurred, accompanied by light production, which was not reversible by simple reductants.[2] The degradation of a ketohydroxy side chain on the luciferin molecule has been presented as a possible explanation of this irreversible reaction.[3] Since purified *Cypridina* luciferin was shown to contain labile phosphate, it has been postulated that the energy derived from the breakdown of the side chain is conserved as phosphate bond energy.[4] This suggestion accounts satisfactorily not only for the irreversible reaction observed *in vitro* but also for the energy requirements of luminescence. The hypothesis was supported by the observation that phosphate was released during the luminescent reaction.

Recently results have been obtained with Lampyrid beetles, popularly known as "fireflies," which support the early suggestion that labile phosphate is concerned in the luminescent reaction. When live fireflies (*Photinus pyralis*) are ground with sand and water in a mortar one obtains momentarily a preparation which is highly luminous; however, the light disappears rapidly with continued grinding. Presumably the non-luminous extract contains the enzyme luciferase, and the luciferin which has been irreversibly decomposed. However, if one adds a small amount of adenosine triphosphate (ATP)[5] to this crude extract a brilliant flash of light appears immediately and lasts for a considerable time depending on the ATP concentration. The results summarized in figure 1 showing the

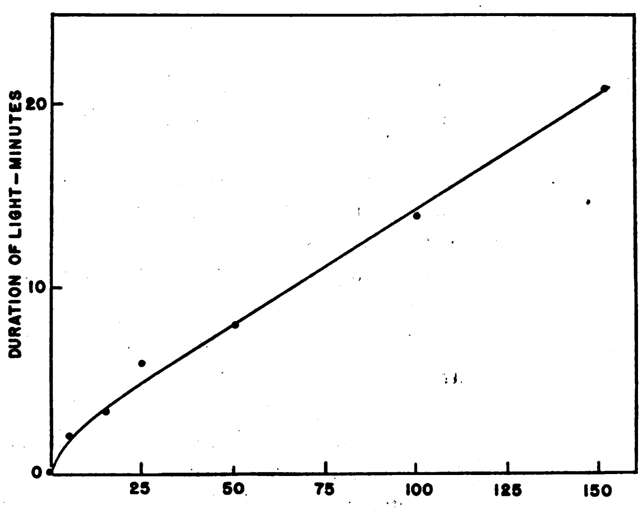

FIGURE 1

The relationship between light emission and adenosine triphosphate

relationship between ATP concentration and duration of light were obtained with the following preparations. The fireflies were placed for 30 minutes in absolute ethyl alcohol containing dry ice and then dried under vacuum in the frozen state. After drying, the lanterns were removed from 50 fireflies and ground with sand and 15 ml. of cold water for 10 minutes. The preparation was centrifuged for 20 minutes (R.C.F. = 2000) and the supernatant, which presumably contained luciferin, was saved. To obtain the enzyme 50 live fireflies were ground with sand and 50 ml. of water and finally centrifuged as above and the supernatant saved. When the two extracts were combined no light appeared. However, with the addition of ATP the entire preparation gave what appeared to be a very homogeneous glow. In the above experiments 0.1 ml. of the substrate was mixed with 0.1 ml. of enzyme solution. Water or ATP was added to give a final volume of 0.7 ml.

At the present time it is not possible to measure the total amount of light emitted by a given concentration of ATP. Therefore, one cannot make a quantitative comparison between light and ATP. With low concentrations of ATP a very bright light is first observed which then decays slowly and finally disappears. With higher concentrations of ATP a bright light is maintained fairly constant for a while and then decays slowly. Consequently, with low concentrations of ATP which maintain the light for only a few minutes a large percentage of the observation time is concerned with the decay part of the curve in contrast to higher concentrations in which the decay portions of the curve represent only a small percentage of the total time.

In the above preparation it was observed that the luciferase preparation itself could be made to luminesce if ATP were added to it. However, the alcohol-treated preparation did stimulate the luciferase preparation and a much brighter light was obtained. Like *Cypridina,* however, it is possible to dialyze the crude extract and obtain a diffusible, temperature-stable substrate and a non-diffusible temperature-labile substance, which when mixed in the presence of ATP will emit light. The dialyzed luciferase preparation did not emit light when ATP was added. The difficulty of dialyzing the enzyme free of the substrate, however, indicates that the combination is a fairly strong one, resembling certain prosthetic groups and apoenzyme combinations. It is possible to concentrate the dialyzable substance by evaporation, and attempts are being made at the present time to identify the compound or compounds concerned.

Since the above observations were made it has been found that the light emitting system can be concentrated from a fresh aqueous extract of the fireflies simply by drying the extract under vacuum. Likewise, whole flies can be dried under vacuum and an extract made of the dried lanterns. However, if the whole fireflies are kept in the dried state for several days the extracted system cannot be made to luminesce with the addition of ATP even though a bright light is obtained during the extraction procedure. This is in contrast to the dried extract made from fresh material which always emits light with the addition of ATP, even after remaining in the dried state for over a month. It has also been observed that oxygen is necessary for light emission in the firefly extract, for when the various components are mixed under anaerobic conditions no light is obtained. With the admission of air, however, the usual bright glow appears immediately.

It is not possible at the present time to say whether ATP is concerned directly or indirectly with light production. With the crude preparations it is conceivable that ATP is a necessary component for some system which in turn "re-activates" the luciferin. However, the earlier observations that acid-labile phosphate is present in the purified *Cypridina* luciferin preparations and that phosphate is released concomitantly with light emission lead one to suspect that the "energy rich" phosphate groups are directly concerned in exciting the luciferin molecule. On the basis of the previous hypothesis, then, it is suggested that the light-emitting reaction is reversed by the transfer of phosphate from ATP to the luciferin molecule, the energy requirements for light emission being satisfied primarily by phosphate bond energy. This suggests that luciferin may act in both phosphate and electron transporting systems.[6]

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[3] Chakravorty, P. N., and Ballentine, R., *J. Am. Chem. Soc.*, **63**, 2030 (1941).

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[5] The adenosine triphosphate was prepared from rabbit muscle as the barium salt according to the method of Needham, using the modifications given in Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, Burgess Publishing Co., Minneapolis, 1945. According to the 7 minute phosphate and purine analysis the preparation was 85% pure.

[6] I wish to thank Drs. E. N. Harvey, A. M. Chase, R. S. Anderson and Robert Ballentine for suggestions made in the preparation of the manuscript and Dr. John B. Buck for identifying the species of fireflies.